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A PROBABLE EXPLANATION OF POLYEMBRYONY IN THE ARMADILLO

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By arresting the development of the fish's egg during early stages double individuals and twins are frequently induced. The interruption or arrest makes it possible for more than one potential growth point along the germ-ring to give rise to an embryonic shield. In other words, accessory invaginations or blastopore formations occur as the initial structural step in doubleness. The interruption in the development of the fish embryo must be introduced during the cleavage stages and before gastrulation in order to produce such phenomena. Among hundreds of eggs arrested during later developmental stages no double monsters or twins ever occurred. A complete account of these experiments is soon to be published but for our present purpose two facts are important: First, accessory embryo formations result from arrests in the developmental process; and second, the arrest must occur before gastrulation has taken place.

In the light of these experiments it has seemed possible to interpret somewhat more clearly than has formerly been done the remarkable phenomenon of multiple embryo formation in the armadillo.

On examining the uterus in two pregnant specimens of a South American armadillo von Jhering, in 1885, discovered that each contained eight fetuses enclosed within a single chorion. He correctly concluded that all of the fetuses in each mother had been derived from a single egg by some process of division into separate embryonic rudiments. After this valuable discovery and interpretation, the study of the armadillo's development lapsed and nothing of importance was added for almost twenty-five years. Two series of investigations were then begun simultaneously one on the Texas armadillo by Newman and Patterson,[1] and the other on the South American species by Fernandez.[2] The growth and expansion of these twin studies has brought our understanding of the phenomena of polyembryony in the armadillo to a considerable state of maturity.

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[1] H. H. Newman and J. T. Patterson, *Jour. Morph.*, Vol. 21, p. 359, 1910.

These authors readily agreed that in most species of armadillo the individual members of a litter, usually four in the Texas species and eight in the common South American form, are all derived from a single egg. It required considerable effort, however, to obtain the material that would furnish the morphological stages of the process by which the polyembryonic development was accomplished. We are finally indebted to Patterson,[3] for the very thorough and satisfactory manner in which he has collected and studied the early embryonic conditions; and particularly for having shown the first stages of the budding process through which the single blastocyst gives rise to four distinct embryonic areas, each exhibiting a typical primitive streak region.

In connection with the fish experiment it now becomes important to ascertain exactly what degree of development has been attained by the armadillo blastocyst at the time the budding process begins. And since, according to my interpretation, these buds should arise at the time of gastrulation or blastopore formation, it becomes necessary to consider very briefly the germ-layers and gastrulation in mammals. The decidedly precocious and highly modified method of forming the primary germ-layers in the mammalian blastocyst is not strictly comparable to gastrulation or the method of germ-layer formation found among the other vertebrates. On the other hand, the embryonic line or primitive streak of the mammalian egg is exactly comparable to the blastopore and head process formation in the simpler forms.

The blastocyst of the armadillo has already, by a process of cell migration and delamination, separated off the primary entoderm from the ectoderm and further modified these layers before the budding which forms the embryonic primordia has begun. The primordia are first formed by a thickening of the ectodermal layer of the blastocyst. The primary entoderm then invaginates into the primordia to form the secondary entoderm of the gut. The precocious cell migration and splitting into layers in the mammal's egg is associated with the early implantation of the embryo upon the uterine-wall of the mother, and the later primitive streak formation may be interpreted as related to the actual gastrulation or blastopore formation away from which the line of the embryo always develops.

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[2] M. Fernandez, *Morph. Jahrb.*, Bd. 39, p. 302, 1909.

[3] J. T. Patterson, *Jour. Morph.*, Vol. 24, p. 559, 1913.

Whether the validity of the above briefly outlined interpretation of the germ-layer formation is admitted or not, we have in the armadillo a process of budding taking place from the blastoderm and associated with accessory or extra blastopore formation in much the same way as are the accessory embryos along the germ-ring in the egg of the bony-fish. These buds also accord with Kopsch's description of a double gastrular condition with two blastopores in a blastoderm of Lacerta agilis, from which he concluded that twin formation as well as anterior duplication arises from a double Einstülpungen. And further, Assheton has described a similar condition in a blastodermic vesicle of the sheep. He, however, imagined the condition to have been due to a splitting during the morula stage.

The double primitive streaks in the hen's egg and other forms all lend themselves to strengthen the interpretation that double embryo formation first asserts itself by a double gastrulation or blastopore formation, which is initially a process of double instead of single bud formation. Patterson's description of the origin of the quadruplet buds in the Texas armadillo furnishes the most striking case in the study of these conditions. And we may conclude that the budding or accessory embryo formation in the egg of the armadillo is exactly the same developmental process as that which gives rise to twins and double individuals in other vertebrate eggs.

However, the very important question yet remains to be answered. Why does this accessory bud formation occur so constantly in the Texas armadillo in contrast to the single embryo formation of mammalian eggs in general? Patterson failed to answer this question, but he supplied some very significant data which Newman,[4] has appreciated as being intimately connected with the occurrence of polyembryony.

In connection with the collection of material Patterson[3] discovered a "period of quiescence" of the embryonic blastocyst. Regarding this he states:

> The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to

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[4] H. H. Newman, "The Biology of Twins," Univ. of Chicago Press, 1917.

> obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collection in 1911.

The following year he began collecting still two weeks earlier and again had a similar experience.

> Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area).
>
> It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15) at which I have collected, possesses a free blastocyst. . . . Taking all the facts into consideration, I estimate the "period of quiescence" to last about three weeks; that is, from about the middle of October to the third or fourth of November.

In a study of sections no mitotic divisions were found to occur in the blastocysts during the "quiescent period."

The only point of interest cited by Patterson in connection with this peculiar phenomenon of interruption in development, was the fact that in no other mammal, except the deer, had such a condition been found. Bischoff had long ago, 1854, reported a "period of quiescence" lasting for some weeks during a so-called morula stage of the deer embryo.

Newman[4] has recognized the importance of Patterson's discovery of a "quiescent period" during the early development of the armadillo, and states in a discussion of twin formation that this "period of quiescence" probably, "holds the clue to the physiological explanation of polyembryony." In this position Newman is, in my opinion, largely right, but this is as far as the data led him, and he finally remarks:

> The problem is to locate the factors responsible for the slowing down of the developmental rhythm. Whatever these factors may be, and we have no definite knowledge of them, the result of retardation is polyembryony.

Newman thus fails to appreciate the second point in Patterson's discovery, and that is that the blastocysts always lie free in the uterus during the "period of quiescence." This fact enables us to go one step further since the lack of attachment and, therefore, lack of oxygen supply are very probably "the factors responsible for the slowing down of the developmental rhythm."

The armadillo egg like that of most mammals undergoes its early development in the fallopian tube and is, therefore, capable of reaching the blastocyst stage on its initial oxygen supply. After this time, however, it must become attached to the uterine wall for a further source of oxygen. For some reason in the armadillo the reaction between the blastocyst and the uterine wall is postponed, and the blastocyst is incapable of further developmental progress until this reaction is established and the necessary supply of oxygen becomes available. In exactly the same way the development of the blastoderm in the fish's egg is experimentally retarded or stopped by reducing the available oxygen supply and is again made to resume its development by supplying oxygen. In the case of the fish egg, the supply of ordinary nutriment is certainly not involved, and reactions similar to those of the armadillo egg are only obtained as responses to changes in temperature and rate of oxidation.

In the armadillo egg I also do not believe the retardation is of the nature of a starvation phenomenon, since we see nothing of the kind in other forms. Temperature changes are ruled out, since the temperature of the uterus is more or less constant. The absence of oxygen necessary for the energetic process of cell division, is, therefore, in all probability the arresting cause, and the retardation results in polyembryony.

Thus Patterson has found the developmental interruption to exist, and he has also shown the blastocyst to be disconnected from the uterine wall and its necessary oxygen supply during this time. However, he has furnished no data bearing on the reason for the delay in uterine reaction and the consequent failure of immediate implantation of the blastocyst such as normally occurs in other mammals. However, from what is known of the dependence of uterine reactions on conditions in the ovary (Leo Loeb,[5] Stockard and Papanicolaou[6] and others) it may very probably be that some peculiarity in corpora lutea formation is primarily responsible for the entire series of reactions leading to polyembryony in the armadillo.

The consideration of the armadillo egg up to this point has taken account only of the external factors influencing its mode of development. It must now be remembered as a fact of serious importance that the production of quadruplets from the single egg of the Texas armadillo is an almost constant occurrence, while the experimental attempts to produce twins and double individuals in fish eggs and other forms have given at best only small percentages of such individuals among the large groups of eggs treated. It is also a fact that all eggs do not furnish equally favorable material for artificial twin production. The eggs of the trout seem unquestionably more disposed to give rise to twin formations than do the eggs of Fundulus. Thus some eggs would seem to have a hereditary or truly innate predisposition towards polyembryonic formations. There is much reason to believe that aside from the external factors discussed, the armadillo egg itself is highly disposed toward the formation of accessory embryonic buds.

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[5] Leo Loeb, *Jour. Morph.*, Vol. 22, 1911.

[6] C. R. Stockard and G. N. Papanicolaou, *Am. Jour. of Anat.*, Vol. 22, 1917.

There is the possibility, of course, that this natural experiment with the armadillo egg has become so exactly regulated as to influence the developmental processes precisely the same way each time, yet this is highly improbable. The armadillo egg is not a case of simple twin growths from the blastoderm, but as Patterson finds, there are primarily two buds, and then very promptly two secondary ones arise making the four and after this the budding process ceases. In the South American species, however, it would appear as though a tertiary budding occurred giving the usual eight embryos; and in rare cases still another budding occurs from a few of the existing buds giving a total of as many as twelve. It would certainly seem as though the blastoderm in these species passes through a stage of agametic reproduction or budding of a nature unknown among other higher vertebrates. But the possibility for such expression might only exist on account of the delay in implantation of the blastocyst and consequent shortage of the oxygen supply necessary for the rapid formation and growth of the single embryo.

It is important to keep in mind that there are species of the armadillo which produce only a single offspring from one egg. It is not known whether their embryos have a "period of quiescence" but if they have, the period either occurs at a different developmental stage or the eggs do not possess the inherent budding tendency of the other species.

We have further to acknowledge the fact that although the egg of the deer has a "period of quiescence" during its development it does not give rise with any degree of frequency to twin individuals. In the first place it is entirely uncertain from the scanty accounts as to what time in development the quiescent period occurs. Assuming that such a period does exist, it might occur at some indifferent stage when no peculiar result would be expected, for example after gastrulation, as it does in the bird with no subsequent effect. In the light of the experimental production of double individuals it is readily understood that even though the egg of the deer is interrupted in its development at an early stage, it might still be capable, on resuming development, of giving a normal single embryo. A study of the experi*mental production of twin and double individuals among fish* leads one to be surprised at the case of the armadillo, and to expect the reaction found in the deer. The constant interruption occurring in the development of the birds and other animals at indifferent developmental moments with no subsequent ill effects, renders commonplace the fact that the deer successfully withstands an interruption during its development without noticeable modifications in structural response. A full consideration of the different results following interruptions at critical and indifferent developmental moments will be published in a forthcoming number of the *American Journal of Anatomy.*

In conclusion we may summarize the cases as follows: The development of the armadillo is interrupted on account of a failure to become promptly implanted on the uterus and a consequent exhaustion of the available oxygen supply. The interruption occurs at a critical period just preceding the primitive streak and embryonic line formation. This egg appears to have a decided tendency under conditions of arrest to form accessory embryonic buds. As a result of the interaction of these external and internal forces polyembryony is produced.

In the case of the deer only one probable fact is known, and that is that a "period of quiescence" occurs. It is uncertain at what stage the arrest takes place but it is probably due as in the armadillo to a delayed implantation of the blastocyst. Either on account of the stage of arrest, or a lack of tendency to form accessory embryo-buds a typically single individual arises from this egg. The external factors may be the same as in the case of the armadillo, but they interact with different internal factors or different developmental moments to give a very different result.